

## [10] 5-Fluoroorotic Acid as a Selective Agent in Yeast Molecular Genetics

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Genetic techniques that permit the selection of mutant cells in the presence of large numbers of wild-type cells are extremely useful. These selective techniques usually depend on the conversion of a nontoxic compound to one that is toxic to wild-type cells. Mutant cells lacking the ability to form the toxic compound grow in the presence of the inert precursor. In yeast only a few selective compounds of this type have been discovered:  $\alpha$ -aminoadipate prevents the growth of *LYS2*<sup>+</sup> and *LYS5*<sup>+</sup> cells<sup>1</sup>; methyl mercury prevents the growth of *MET2*<sup>+</sup> and *MET5*<sup>+</sup> cells<sup>2</sup>; and both ureidosuccinate<sup>3</sup> and 5-fluoroorotic acid<sup>4</sup> prevent the growth of *URA3*<sup>+</sup> cells. Spontaneous or induced mutations can be selected in each of these genes by plating large numbers of wild-type cells on the appropriate inhibitor.

The 5-fluoroorotic acid (5-FOA) selection is extremely useful for a number of genetic and molecular biological manipulations requiring the detection of rare *ura3*<sup>-</sup> cells. The utility of the 5-FOA selection is due to several factors: the availability of a large collection of *URA3*-based cloning vectors of various types for yeast; the small size and known sequence of the *URA3* gene<sup>5</sup>; the availability of numerous well-studied mutations in the *URA3* gene; the specificity, ease, and efficacy of the selection.

### *The 5-Fluoroorotic Acid Selection*

Yeast cells are generally pregrown in liquid YPD medium or on YPD plates<sup>6</sup> prior to selection on 5-FOA. As many as 10<sup>7</sup> cells may be plated on a single, 50 mm Petri dish containing 5-FOA medium (Table I). Resistant colonies will grow up within 4–7 days at 30°. The most useful concentra-

<sup>1</sup> B. B. Chattoo, F. Sherman, D. A. Azubalis, T. J. Fjellstedt, D. Mehvert, and M. Ogur, *Genetics* **93**, 51 (1979).

<sup>2</sup> A. Singh and F. Sherman, *J. Bacteriol.* **118**, 911 (1974).

<sup>3</sup> M. Bach and F. LaCroute, *Mol. Gen. Genet.* **115**, 126 (1972).

<sup>4</sup> J. D. Boeke, F. LaCroute, and G. R. Fink, *Mol. Gen. Genet.* **197**, 345 (1984).

<sup>5</sup> M. Rose, P. Grisafi, and D. Botstein, *Gene* **29**, 113 (1984).

<sup>6</sup> F. Sherman, G. R. Fink, and C. Lawrence, "Methods in Yeast Genetics." Cold Spring Harbor Lab., Cold Spring Harbor, New York, 1977.

TABLE I  
5-FLUOROOROTIC ACID  
MEDIUM<sup>a</sup>/2× CONCENTRATE<sup>b</sup>

Ingredient	Amount
Yeast nitrogen base	7 g
5-Fluoroorotic acid <sup>7</sup>	1 g
Uracil	50 mg
Glucose	20 g
H <sub>2</sub> O	to 500 ml

<sup>a</sup> Add ingredients to 500 ml 4% agar (autoclaved). Mix and pour into Petri dishes.

<sup>b</sup> Heat to 65° to dissolve and filter sterilize.

tion of the drug is generally 1 mg/ml 5-fluoroorotic acid,<sup>7</sup> but in the interest of economy it is possible to use as little as 500  $\mu$ g/ml 5-FOA. When lower concentrations of 5-FOA are used, however, background growth of the Ura<sup>+</sup> cells is considerably greater. This background growth is less of a problem when Ura<sup>-</sup> derivatives occur at a high frequency (e.g., when a *URA3*<sup>+</sup> marker is located between the elements of a direct duplication). In some instances it may be desirable to use a rich medium such as YPD as opposed to the minimal medium normally used with 5-FOA selection. Unfortunately, addition of 5-FOA to YPD medium at 1 mg/ml prevents the growth of some Ura<sup>+</sup> strains but not others, whereas the 5-FOA minimal medium in Table I inhibits all Ura<sup>+</sup> yeast strains we have tested. The reason for the variability on YPD is not clear. On the other hand, the addition of 5-FOA at 1 mg/ml to SC medium,<sup>6</sup> which contains all 20 amino acids and other supplements added to minimal medium, gives satisfactory results with all of the strains we have tested.

#### *Transplacement of Mutant Alleles: Use of 5-Fluoroorotic Acid*

Selection of Ura<sup>-</sup> cells with 5-FOA is used frequently in the replacement of resident chromosomal genes by transformation.<sup>8</sup> Generally the goal is to replace the gene of interest with a mutant allele of that gene generated *in vitro* (Fig. 1). Replacement of the wild-type gene with a deletion mutation permits the assessment of the effect of a null mutation on the phenotype of the cells. If there is a possibility that the gene is

<sup>7</sup> Available from Pharmacia/P-L Biochemicals, Piscataway, New Jersey, and SCM Specialty Chemicals, Gainesville, Florida.

<sup>8</sup> F. Winston, F. Chumley, and G. R. Fink, this series, Vol. 101, p. 211.

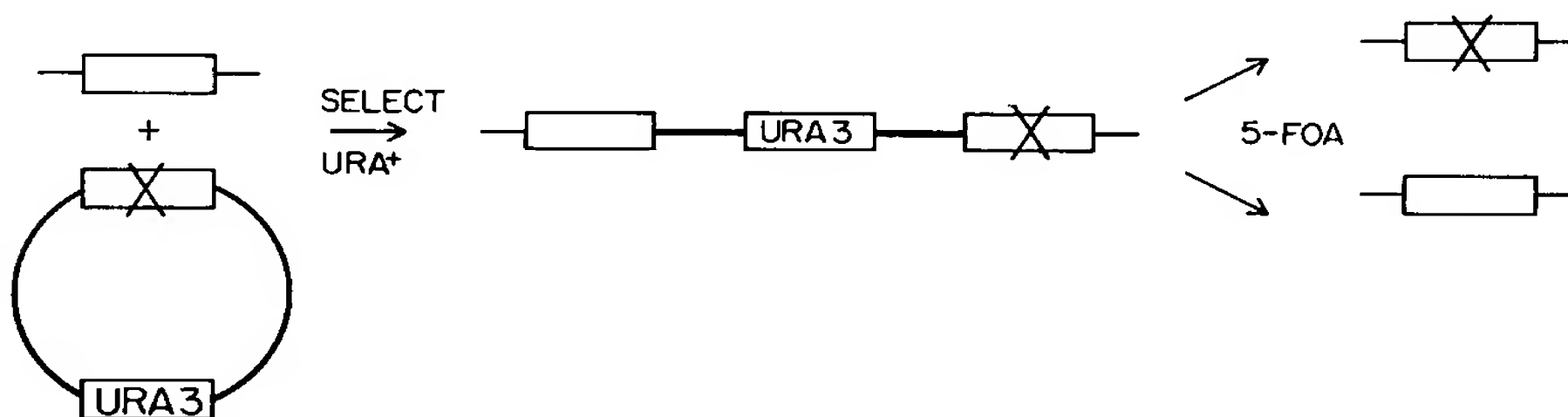


FIG. 1. Transplacement of a yeast gene with a mutant allele. The heavy lines indicate pBR322 sequences. The X symbolizes a mutation introduced into the cloned DNA. The gene of interest (open box) is cloned into Yip5, a yeast integrating vector carrying the *URA3* gene as the selectable marker. The plasmid is used to transform a yeast strain containing a nonrevertable *URA3* allele (e.g., *ura3-52*) to *Ura*<sup>+</sup>. The structure of the chromosome in the *Ura*<sup>+</sup> transformant is shown in the center: the *URA3* gene and pBR322 sequences are flanked by the wild-type (left) and mutated (right) versions of the gene. When the cells containing the integrated plasmid are plated onto medium containing 5-fluoroorotic acid (5-FOA), derivatives which lose the *URA3* gene and the flanking plasmid DNA (*Ura*<sup>-</sup>) via a homologous recombination event between the two homologous segments flanking *URA3*<sup>+</sup> survive, whereas the parent cells (*Ura*<sup>+</sup>) do not.

essential, these experiments are normally performed in a diploid homozygous for a nonreverting *ura3* mutation such as *ura3-52*. If the gene in question is known to be nonessential, the experiments can be performed in the corresponding haploid strain. The deletion mutation is introduced in a derivative of Yip5<sup>9</sup> or a similar yeast integrating plasmid bearing both the *URA3* gene and the gene of interest. Transformation of the target strain with the deleted Yip5 derivative by either the spheroplast<sup>10</sup> or lithium acetate<sup>11</sup> procedures, followed by selection for the *Ura*<sup>+</sup> phenotype, results in integration of the mutant form of the gene in a tandem arrangement with the wild-type allele (Fig. 1). The *URA3* gene and plasmid sequences lie between the duplicated sequences in the transformants, which are referred to as integrants.

The next step in the allele replacement process is a selection for cells that have undergone a reversal of the integration process (this reversal may be viewed as a single-crossover recombination event). This step is greatly facilitated by the use of 5-FOA. Cells which undergo the desired recombination event will lose the plasmid and *URA3* sequences, thereby

<sup>9</sup> D. Botstein, S. C. Falco, S. E. Stewart, M. Brennan, S. Scherer, D. T. Stinchcomb, K. Struhl, and R. W. Davis, *Gene* 8, 17 (1979).

<sup>10</sup> A. Hinnen, J. B. Hicks, and G. R. Fink, *Proc. Natl. Acad. Sci. U.S.A.* 75, 1929 (1978).

<sup>11</sup> H. Ito, Y. Fukuda, K. Murata, and A. Kimura, *J. Bacteriol.* 153, 163 (1983).

becoming resistant to 5-FOA (a procedure variously referred to as “popping out,” “looping out,” or excision of the plasmid). The fraction of popouts which leave behind the mutant allele will depend on the relative recombination frequencies of the two segments of DNA flanking the mutation. (This frequency is usually related to the size of the homology regions flanking the mutation.) If a particular phenotype is expected or predicted, the presence of the mutant allele can be ascertained by phenotypic screening. Where one does not know what phenotype to expect, the mutant allele may be identified by Southern hybridization to the chromosomal DNA from several 5-FOA<sup>r</sup> derivatives in order to identify the mutant allele. This analysis assumes that the allele has some characteristic restriction site or pattern on a gel.

*Plasmid Shuffling: A General Technique for Isolating Conditional Mutations in an Essential Gene*

Essential genes present special problems in mutational analysis because their deletion is lethal. To obtain viable cells with nonfunctional alleles of an essential function, investigators have turned to temperature-sensitive alleles. A typical mutant hunt for such alleles involves the identification of strains that fail to form colonies at the restrictive temperature, but allow normal growth at the permissive temperature. If the gene has been cloned, then one can mutagenize the cloned gene *in vitro* and transform it back into yeast. The best recipient would be a strain containing a deletion of the gene on the chromosome so that the alleles produced on the *in vitro* mutagenized copy will be revealed after transformation. The recipient strain must possess a functional copy of the gene until it obtains a second copy introduced on the mutagenized plasmid. However, a functional copy of the gene will mask the phenotype of any temperature-sensitive allele borne by a plasmid. The resident gene must then be lost or destroyed so that the function of the mutagenized gene may be assayed. This Byzantine conundrum has several solutions.

One solution involves transformation of a wild-type strain with a mutagenized integrating plasmid that carries a truncated version of the cloned gene.<sup>12</sup> The appropriate integration event leads to disruption of the wild-type chromosomal copy and restoration of the missing sequences to the mutagenized copy (Fig. 2A). The only intact copy of the gene contains mutagenized sequences, so if a mutation has been induced, the phenotype corresponding to that mutation will be expressed even if it is recessive. This technique suffers from the constraints imposed on the integration

<sup>12</sup> D. Shortle, P. Novick, and D. Botstein, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4889 (1984).

reaction. First, integrative transformation is a low frequency event. Second, if the crossover occurs on the incorrect side of the mutation, or if gene conversion by the wild-type allele accompanies the integration, the mutation may not be recovered (Fig. 2B, C). Moreover, in at least one study<sup>12</sup> the majority of temperature-sensitive mutations obtained by this procedure did not map to the gene in question. Experiments using this procedure in our laboratory also gave a high proportion of unlinked temperature-sensitive mutations. Apparently, the transformation process itself has a general mutagenic effect on the recipient cell.

We devised a plasmid shuffling technique to obviate the problems in obtaining temperature-sensitive mutations in the *DLF* (desired lethal function) gene. Mark Rose was extremely helpful in the development of these ideas. The procedure requires construction of a *ura3<sup>-</sup> leu2<sup>-</sup>* recipient containing a lethal deletion of *DLF* ( $\Delta dlf$ ) on the chromosome and a wild-type copy (*DLF<sup>+</sup>*) on a *URA3<sup>+</sup>* plasmid. This strain is then transformed by a second plasmid containing the selectable *LEU2<sup>+</sup>* marker and a copy of the *DLF<sup>+</sup>* gene. The *LEU2* plasmid is mutagenized *in vitro* prior to transformation. The mutations induced in the *DLF<sup>+</sup>* gene on the *LEU2* plasmid are revealed when the *DLF<sup>+</sup>* gene on the *URA3<sup>+</sup>* plasmid is lost upon 5-FOA selection, because the cells then have only a deleted  $\Delta dlf$  gene on the chromosome. The actual manipulations will be described in subsequent sections.

The plasmid shuffling strategy has been used successfully to obtain temperature-sensitive mutations in the *CDC27* gene. Previously isolated

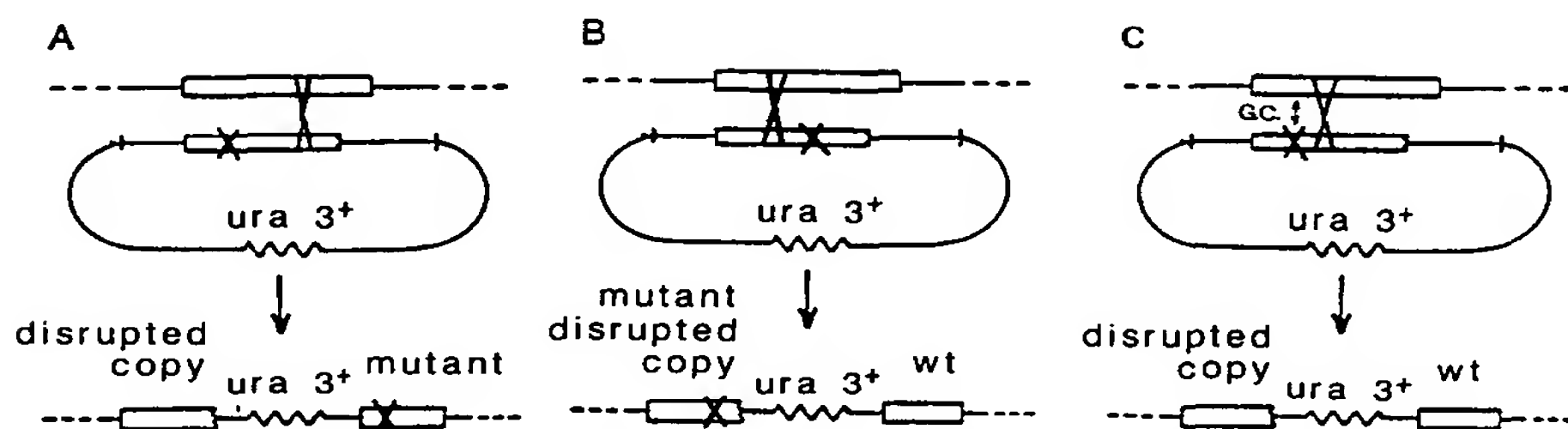


FIG. 2. Integration of a mutagenized, truncated gene. An integrating plasmid is constructed bearing a truncated version of the gene of interest. The gene can be deleted at either the 5' or 3' end. If a mutation is induced in the truncated segment, and integration occurs as in (A), the complete copy of the gene will harbor the mutation. However, if the integration occurs as in (B), the complete copy of the gene will not contain the mutation. If the integration is accompanied by gene conversion (GC) of the mutated segment back to its wild-type state, the mutation will not be recovered (C).



temperature-sensitive mutations in *cdc27* cause the cell to arrest late in nuclear division upon growth at the elevated temperature.<sup>13</sup> We have mapped this gene to the left arm of chromosome II, 13 centimorgans distal to *ils1*. Sporulation and dissection of a diploid containing a wild-type *CDC27* gene and a disrupted *cdc27Δ1* gene yielded two viable and two inviable spores per tetrad. When the same diploid was transformed with pSB17, a 2μ-based plasmid carrying both the *URA3* and the *CDC27* gene, seven out of seven tetrads tested gave four viable spores. Moreover, in each of the seven tetrads two of the spores failed to segregate 5-FOA-resistant derivatives, an indication that strains containing the deletion cannot survive without the *URA3<sup>+</sup> CDC27<sup>+</sup>* plasmid. Southern analysis confirmed the presence of the *cdc27Δ1* allele in the chromosomes of these strains. This experiment shows that at least one copy of *cdc27* is required for growth.<sup>14</sup>

The plasmid shuffle required a second plasmid carrying *CDC27* on a centromere vector which contained the *LEU2* gene as the selectable marker. The *CDC27<sup>+</sup>* gene on this vector is mutagenized by hydroxylamine and introduced by transformation into a strain containing a disrupted chromosomal *cdc27Δ1* gene and an intact episomal *CDC27* gene on a *URA3*-2μ vector. *Leu<sup>+</sup>* transformants were selected on plates containing uracil but lacking leucine. This combination of nutrients selects for the presence of the mutagenized *LEU2*-*CDC27* plasmid, and simultaneously releases any selection for the *URA3*-*CDC27* plasmid. These transformants are grown at the permissive temperature, during which time a proportion of the transformed cells lose the unmutagenized *CDC27<sup>+</sup>* gene carried on the *URA3* plasmid. These *Ura<sup>-</sup>* cells are now capable of expressing only the phenotype of the *CDC27* gene on the *LEU2* plasmid. After the transformed colonies are replica-plated onto 5-FOA medium, one can assay the properties of these *Ura<sup>-</sup>* cells. If the *LEU2* plasmid carries a nonmutant *CDC27<sup>+</sup>* gene, the *Ura<sup>-</sup>* cells will be able to grow at both permissive and restrictive temperatures. Since the original colony is a mixture of *Ura<sup>+</sup>* and *Ura<sup>-</sup>* cells, the replica on 5-FOA will give rise to *Ura<sup>-</sup>* papillae that grow out of the background. If, on the other hand, the *LEU2* plasmid bears a temperature-sensitive *cdc27* gene, the *Ura<sup>-</sup>* cells within the colony will be able to grow at the permissive but not the restrictive temperature. A null mutation in the *LEU2* plasmid-borne gene will render any *Ura<sup>-</sup>* segregant inviable: no papillation of 5-FOA<sup>r</sup> colonies will be seen at any temperature (Fig. 3).

<sup>13</sup> L. H. Hartwell, R. K. Mortimer, J. Culotti, and M. Culotti, *Genetics* **74**, 267 (1973).

<sup>14</sup> J. Trueheart, unpublished results.

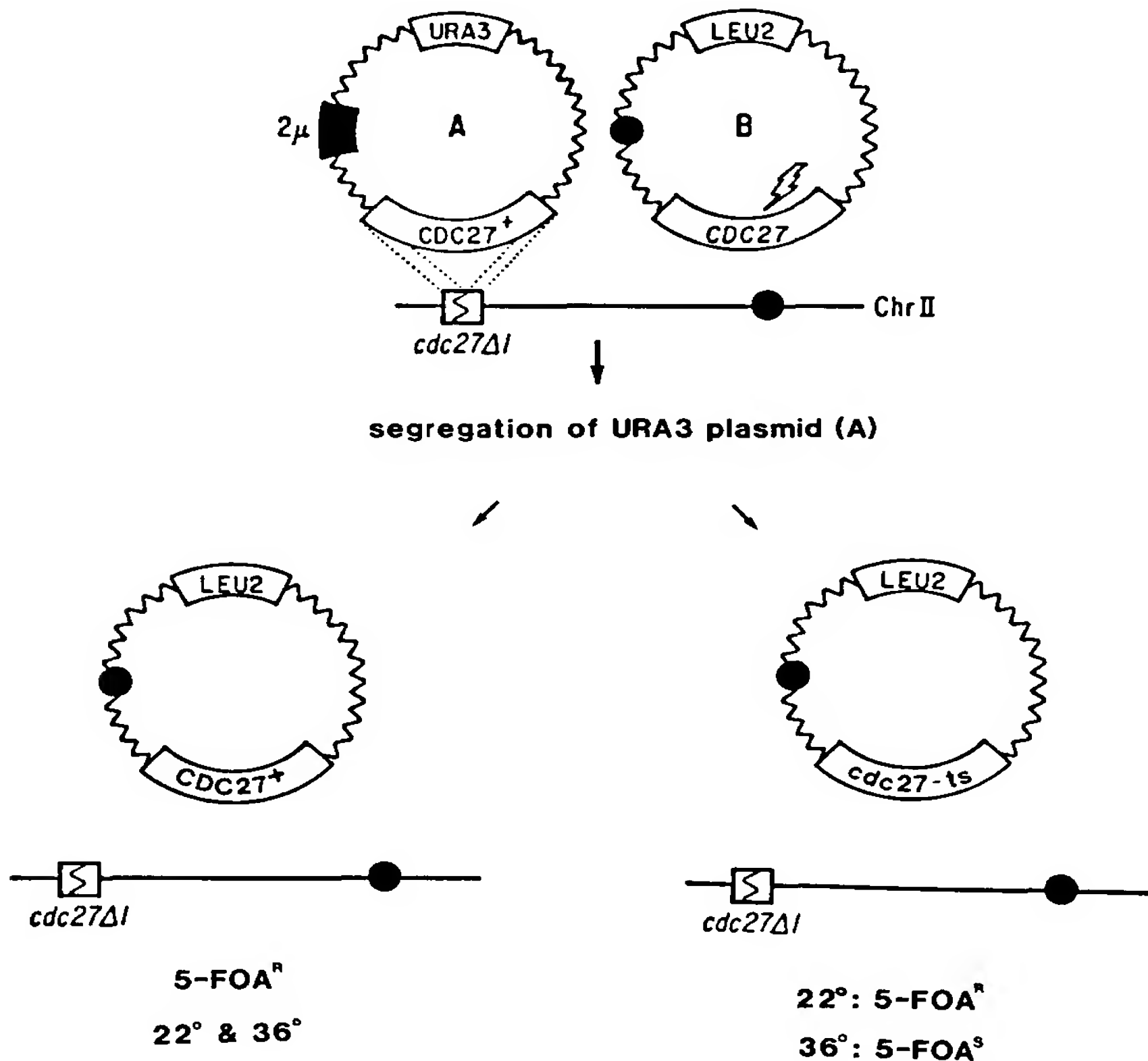


FIG. 3. Plasmid shuffling: a general method for introducing mutations into a cloned essential gene. A strain containing the *cdc27Δ1* allele integrated into chromosome II (solid circles represent centromeres) can be propagated if Yep24 (which carries the *URA3* gene as its selectable marker) bearing the *CDC27* gene (plasmid A) is present in the cell. Because of the *cdc27Δ1* deletion these cells depend on the Yep24 derivative for the essential *CDC27* function. A second, mutagenized plasmid containing the *CDC27* gene and the *LEU2* selectable marker (plasmid B) is subsequently introduced into the same cell. If the *LEU2* plasmid (B) carries a functional *CDC27* gene, the Yep24 derivative (A) becomes dispensable to the cell; 5-FOA-resistant derivatives arise frequently which lack the Yep24 derivative. If the *LEU2* plasmid carries a mutation which inactivates the *CDC27* gene, the cell remains dependent on plasmid A and cannot papillate 5-FOA-resistant derivatives. Any conditional defect in the *CDC27* gene on plasmid B, such as temperature sensitivity, will be reflected in the 5-FOA phenotype, i.e., cells with a temperature-sensitive *cdc27* allele will form 5-FOA<sup>R</sup> papillae only at the permissive temperature.

TABLE II  
PLASMID SHUFFLING: MUTAGENESIS OF THE *CDC27* GENE

Mutagenized plasmids tested	<i>cdc27</i> nonconditional	<i>cdc27</i> temperature sensitive
800	11	2

Strain JY72 [*MATa*, *ura3-52*, *leu2-3,112*, *trp1Δ1*, *cdc27Δ1* (pSB17 =  $2\mu$ -*URA3*-*CDC27*)] was transformed with 9  $\mu$ g of hydroxylamine-mutagenized<sup>15</sup> pSB31 (*LEU2*-*CEN4*-*CDC27*) DNA, giving approximately 800 *Leu*<sup>+</sup> colonies after incubation of the transformed cells at 22° for 4 days. These colonies were replica-plated onto 5-FOA plates with added uracil and tryptophan, and incubated at 36° for 1 day. The phenotypes of the colonies obtained are summarized in Table II. Of 35 colonies that initially failed to papillate 5-FOA-resistant derivatives at high temperature, eleven failed to grow on 5-FOA at any temperature; these were presumed to carry completely nonfunctional copies of the *CDC27* gene. Two isolates displayed the desired phenotype: ability to grow on 5-FOA at 22° but not at 36°, and no growth defect on rich or leucine-deficient media at either temperature. The remaining colonies either failed to retest or displayed an intermediate phenotype at the high temperature upon retesting.

Any temperature-sensitive mutation that arises in an unlinked chromosomal gene can be identified easily and discarded at the beginning of the experiment. Such mutants would fail to grow at the high temperature regardless of the media employed. In the *CDC27* experiment, none of the 35 isolates showed any growth defect on rich or leucine-deficient media at either temperature. Therefore, this strategy seems not to suffer the high rate of unlinked mutation which plagues the integrative technique.

The two putative temperature-sensitive mutants were analyzed further with respect to their cell division cycle (*cdc*) phenotype. Both were cured of pSB17 by growth on YPD medium at 22°, and *Ura*<sup>-</sup> colonies were identified by replica plating to SC-Ura medium. When a segregant from each isolate was grown at 36° in YPD medium, both temperature-sensitive mutants arrested as a uniform population of large-budded cells, as do other previously identified alleles of the *CDC27* gene.<sup>13</sup> This analysis shows that the temperature sensitivity of papillation to 5-FOA resistance observed initially was not merely an artifact stemming from the presence of 5-FOA, but that the drug merely prevented the growth of those cells that still contained the original wild-type gene.

<sup>15</sup> M. Rose and G. R. Fink, *Cell* **48**, 1047 (1987).



*Introduction of ura3 Deletions into the Chromosome*

Because of the widespread use of *URA3*<sup>+</sup> as a selectable marker, the availability of *ura3*<sup>-</sup> deletion mutations of defined structure would be extremely useful. Large deletions of this region do not exist in the current mutant collection. Apparently, deletion of the entire 1.1 kb *HindIII* fragment on which the *URA3* gene resides is lethal to the cell.<sup>16</sup> There is circumstantial evidence that an essential gene lies 5' to the *URA3* gene.<sup>17</sup> The *ura3-52* mutation is nonreverting, but its utility is compromised. The mutation is caused by the insertion of a Ty element and the complete wild-type *URA3* sequences are all still present.<sup>18</sup>

As a first step to constructing a series of defined mutations in the *URA3* gene, we have made a 220-bp deletion extending from the *PstI* site just 5' of the *URA3* ATG initiation codon to the *NcoI* site at position 432.<sup>5</sup> The mutant allele was constructed in an integrating plasmid containing the *TRP1* gene as a selectable marker (the structure of the resultant plasmid outlined in Fig. 4 was confirmed by restriction enzyme digestion). The 5-FOA selection was invaluable in replacing *URA3*<sup>+</sup> with the deletion of *ura3* that we made *in vitro*.

We tested two methods of introducing the *ura3Δ1* allele into yeast cells, both of which make use of the 5-FOA selection. One involved an attempt to introduce the deletion mutation directly by transforming with a linear piece of DNA carrying the deletion and homologous to *URA3* at both ends, followed by direct selection on 5-FOA. The second was to integrate the *ura3Δ1* mutation (using the *TRP1* gene as a selectable marker) at the *URA3* locus and subsequently to select for popouts of the wild-type allele by growth on 5-FOA medium. Only the second method gave the desired insertion of the *ura3Δ1* gene.

Yeast cells (strain JBX169-10B; *MATa*, *trp1Δ1*, *lys2*, *GAL*<sup>+</sup>) were prepared for transformation by the lithium acetate<sup>11</sup> procedure. The cells were transformed with plasmid pJEF1332 (Fig. 4) which had been cleaved with one of two restriction enzymes: *HindIII*, which releases the *URA3* fragment completely from the plasmid, and *StuI*, which cuts within the *URA3* segment but does not cut the plasmid elsewhere. When the *HindIII*-cut plasmid is used in transformation, one expects direct gene replacement<sup>19</sup> of the *URA3* gene on the chromosome by the deletion allele. Presumably the *ura3Δ1* transformants could then be selected on 5-FOA. However, it seemed possible that the cells would require a period

<sup>16</sup> F. Winston and T. Donahue, personal communication.

<sup>17</sup> M. Rose and J. Cappello, personal communication.

<sup>18</sup> M. Rose and F. Winston, *Mol. Gen. Genet.* **193**, 557 (1984).

<sup>19</sup> R. J. Rothstein, this series, Vol. 101, p. 202.

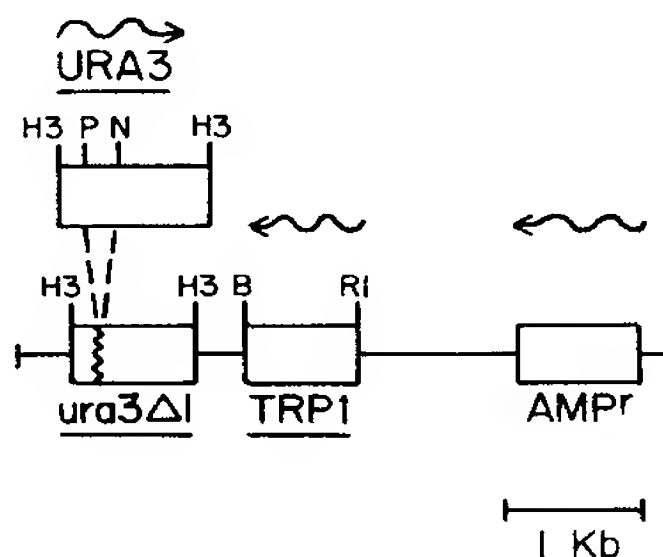


FIG. 4. An integrating plasmid bearing the *ura3Δ1* mutation, pJEF1332. The *ura3Δ1* mutation and its wild-type counterpart are diagramed. Boxes represent genes; wavy lines are transcripts. Restriction sites are abbreviated as follows: H3, *Hind*III; P, *Pst*I; N, *Nco*I; B, *Bam*HI; RI, *Eco*RI. The plasmid backbone (all sequences to the left of *URA3* and to the right of *TRP1*) is from pUC18, drawn as though linearized at position 1 (as defined in the New England BioLabs 1985/86 catalog). The polylinker sequences between *Hind*III and *Eco*RI have been substituted with the yeast genes; the short *Hind*III–*Bam*HI fragment between the *TRP1* and *URA3* genes is derived from pBR322.

of nonselective growth in order for the *Ura*<sup>−</sup> (5-FOA<sup>r</sup>) phenotype to be expressed. Therefore, the cells were cultured in liquid YPD medium overnight prior to plating them onto 5-FOA medium. The results of this experiment were rather disappointing. Although there were about 3 times as many 5-FOA<sup>r</sup> colonies on plates where *ura3Δ1* DNA was added as on control plates to which no DNA was added, the presence of colonies on control plates suggested that spontaneous *ura3*<sup>−</sup> mutants had arisen in the culture without added DNA. Nevertheless, 12 5-FOA<sup>r</sup> colonies from the plate to which DNA had been added were analyzed by Southern hybridization in order to examine the structure of the *URA3* locus. All 12 strains contained the wild-type 1.1-kb *Hind*III fragment rather than the expected 0.9-kb mutant *Hind*III fragment corresponding to the deleted *URA3* gene. The presence of only the wild-type *URA3* fragment suggested that none of the 12 5-FOA<sup>r</sup> colonies resulted from direct replacement by the deletion allele. Probably, each carried some spontaneously arising point mutation in the *URA3* gene. Similar results were obtained in another laboratory in experiments designed to replace the *URA3* locus with cloned DNA derived from the Ty element insertion allele *ura3-52*.<sup>20</sup>

The alternative approach was to introduce the deletion mutation by the integration–excision or popout technique described earlier. Linearized pJEF1332 plasmid was integrated by selection for the Trp<sup>+</sup> phenotype and then segregants that had lost the wild-type *URA3* gene, the

<sup>20</sup> R. J. Rothstein, personal communication.

plasmid sequences, and the *TRP1* gene were obtained on 5-FOA medium. Yeast cells (JBX169-10B) were grown in YPD and transformed with 1  $\mu$ g of pJEF1332 DNA previously linearized with the restriction enzyme *StuI*, which cuts only once in the plasmid, within the remaining coding sequences of the *URA3* gene. The transformed cells were plated on SC – Trp medium in order to select for integration. Five transformants were chosen for further study. Several 5-FOA<sup>r</sup> segregants were isolated from each transformant by simply streaking the transformant colony onto 5-FOA medium (containing tryptophan and other nutritional requirements of the strain). Of 30 5-FOA<sup>r</sup> derivatives, 27 were Trp<sup>+</sup> and 3 were Trp<sup>–</sup>. All 3 Trp<sup>–</sup> derivatives proved to be simple popouts of the pJEF1332 plasmid as judged by Southern analysis and by reversion analysis (as expected, no reversion of the deletion mutation was observed, even after extensive UV irradiation). Presumably, the Trp<sup>+</sup> derivatives resulted from gene conversion of the wild-type copy of the *URA3* gene by the deleted copy. Why this event should be so much more frequent than the popping out of the plasmid, which requires only a single crossover event, is unclear.

The direct selection of Ura<sup>–</sup> using 5-FOA in transformation needs further study, in view of a recent report of the successful use of this procedure. A plasmid containing the *Bam*HI fragment of *URA3*, which is 5 kb in length, was used as the starting material for the construction of a 446 bp deletion which removes the 3' portion of the *URA3* coding sequences (extending from the *StuI* site to the *SmaI* site).<sup>21</sup> Apparently, transformation of Ura<sup>+</sup> yeast with *Bam*HI-linearized plasmid DNA results in 10 to 50 5-FOA<sup>r</sup> transformants per microgram, all of which carry the deletion.<sup>21</sup> Perhaps the increased homology to chromosomal DNA in such an experiment allows for a higher frequency of transformants. No outgrowth in YPD was used in these experiments.<sup>21</sup>

### *Additional Applications*

The 5-FOA selection for Ura<sup>–</sup> cells is very useful in the study of repeated sequences and their relative rates of recombination. It is a simple matter to measure the mitotic recombination frequency of any yeast duplication using 5-FOA. The sequence is simply cloned into Yip5 and then integrated by cutting within the region of homology to the sequence of interest, with selection for the Ura<sup>+</sup> phenotype. The frequency of Ura<sup>–</sup> segregants (detected as 5-FOA<sup>r</sup> colonies) can then be easily measured. Winston *et al.*<sup>22</sup> used the selection to obtain accurate estimates of the rate

<sup>21</sup> K. Frohlich, personal communication.

<sup>22</sup> F. Winston, D. T. Chaleff, B. Valent, and G. R. Fink, *Genetics* 107, 179 (1984).

of  $\delta$ - $\delta$  recombination in Ty elements by inserting the *URA3*<sup>+</sup> marker between the  $\delta$  elements.

### *Summary*

5-FOA is an extremely useful reagent for the selection of Ura<sup>-</sup> cells amid a population of Ura<sup>+</sup> cells. The selection is effective in transformation and recombination studies where loss of *URA3*<sup>+</sup> is desired. A new plasmid shuffling procedure based on the 5-FOA<sup>R</sup> selection permits the recovery of conditional lethal mutations in cloned genes that encode vital functions.

### *Acknowledgments*

J.D.B. was supported by a Helen Hay Whitney Fellowship, G.N. is a fellow of the Belgian Fonds National de la Recherche Scientifique, G.R.F. is an American Cancer Society Professor.

## [11] Transposon Tn5 Mutagenesis to Map Genes

By FRANS J. DE BRUIJN

### *Introduction*

Transposable (Tn) elements (or transposons) are extremely useful tools in both classical as well as molecular bacterial genetics. Their utility is based on a number of specific characteristics:

1. Transposition (or insertion) of a Tn element into a structural gene or its regulatory region usually leads to insertional inactivation of the gene, and these mutations are stable.
2. Tn elements carry genetic markers, such as antibiotic resistance genes, which greatly facilitate genetic mapping of Tn element-induced mutations, as well as the transduction and cloning of mutated regions.
3. Tn element-induced insertion mutations are generally nonleaky, highly polar on genes located downstream in operons, and revert (by precise excision) at very low frequencies.
4. Tn elements usually have a characteristic physical structure, including inverted repeats of varying lengths as well as unique restriction endonuclease cleavage sites, which facilitate mapping of their insertion